The effect of isovolaemic haemodilution and intravenous glycerol on the sequelae of middle cerebral artery occlusion in the rat

A DIETIS, S EHTESHAMI, MJG HARRISON, NI PERINPANAYAGAM

From the Department of Neurological Studies, The Middlesex Hospital Medical School, London, UK

SUMMARY The therapeutic potential of isovolaemic haemodilution and osmotherapy on the sequelae of middle cerebral artery occlusion has been studied in an animal model. The territory of the middle cerebral artery that failed to fill with a carbon black intravascular marker after intracranial occlusion for 30 minutes in the anaesthetised rat was reduced by 50% by isovolaemic haemodilution to a haematocrit of 30%. An intravenous infusion of 10% glycerol prevented swelling of the ischaemic hemisphere, though it did not further improve vascular perfusion. These findings support the use of isovolaemic haemodilution and osmotherapy in the acute aftermath of stroke.

An elevated haematocrit appears to be a risk factor for stroke^{1,2} and to be associated with a higher mortality following acute stroke.³ In a retrospective study of patients with an acute neurological deficit associated with angiographically proven carotid occlusion, the size of their infarct on CT scans proved to be related to prevailing haematocrit.⁴ Since cerebral blood flow is inversely related to haematocrit, it is possible that reduced flow in patients with high haematocrit might adversely affect collateral flow at the time of development of a cerebral infarct, and thereby influence its size and sequelae. Isovolaemic haemodilution has duly been proposed as a possible therapeutic approach.⁵

The major early mortality after ischaemic stroke is due to herniation secondary to swelling of the infarcted hemisphere,⁶ and local oedema is also believed to adversely affect vascular perfusion.⁷ Corticosteroids have proved ineffective in clinical trials,⁸ even in massive doses,⁹ but there is some evidence that hyperosmolar agents such as glycerol, may be more successful.¹⁰

We have therefore investigated the possibility that isovolaemic haemodilution and the use of glycerol might improve vascular perfusion after middle cerebral artery occlusion.

Address for reprint requests: Dr MJG Harrison, Middlesex Hospital Medical School, Mortimer St, London W1N8AA, UK.

Received 30 April 1985 and in revised form 18 July 1985. Accepted 27 July 1985.

Materials and methods

Adult male Wistar rats weighing between 317 and 550 grams were anaesthetised with a single intraperitoneal injection of pentobarbitone ('Sagatal' May & Baker Ltd, 60 mgm/kg). The animals were given a tracheostomy and a femoral artery cannula was inserted for monitoring of arterial haematocrit, mean arterial blood pressure, arterial pCO₂, pO₂ and pH. A femoral venous catheter was also inserted for infusions. Haematocrit levels were measured in a Hawksley micro-haematocrit centrifuge. After establishing physiological baseline values for blood pressure, pCO₂ and pO₂, the zygosoma was removed and access to the right middle cerebral artery obtained through a limited craniotomy as described by Tamura *et al.*¹¹ In some animals isovolaemic haemodilution was achieved by exchanging 3.3 ± 0.15 ml of blood in 0.3 ml aliquots by a 5% albumin suspension. Other animals were studied at normal haematocrit. The middle cerebral artery was then occluded by diathermy and a clip placed on the ipsilateral common carotid artery in the neck. Some animals then received either normal saline or 10% w/v glycerol in saline (3.3 ml/kg) as an intravenous infusion over 30 minutes. At the end of this period an intravenous injection of 2 ml of an iso-osmotic carbon black suspension was given immediately prior to sacrifice while the carotid clip was still in place. The brain was then removed and thick coronal slices cleared in Oil of Wintergreen (fig). The area of the middle cerebral artery territory that failed to fill with carbon particles was measured on each slice using a "digi-plan" microprocessor. The coronal slices were numbered anterior (-ve) and posterior (+ve) to the slice of largest area so that comparisons could be made between different brains. The area of non-perfusion was expressed as a percentage of the area of its own hemisphere.

Fig Coronal slice of rat brain after iv. infusion of a carbon black suspension to demonstrate the area of non-filling of the vascular bed after occlusion of the middle cerebral artery.

The whole area of the hemisphere was also recorded to assess any swelling of the ischaemic hemisphere.¹² This technique was preferred to the measurement of wet weight/dry weight difference or specific gravity measurements so that some idea of the presence of swelling could be obtained from the same specimens that were being processed for calculation of extent of vascular perfusion.

If blood pressure fell at any stage in the protocol to below 75% of the initial level, the experiment was terminated. No change in blood pressure occurred with arterial occlusion or with glycerol infusion.

Results

Isovolaemic haemodilution

One animal became hypotensive during the exchange of blood and albumin, and was excluded from further consideration. There remained five animals in whom the haematocrit was reduced to $30.4 \pm 1.1\%$, and six animals not exchanged whose mean haematocrit was

$52.3 \pm 1.6\%$ ($p < 0.001$, Student *t* test). The exchanged animals had slightly lower pO_2 but there was no significant change in pCO_2 or pH (table 1). The area of the middle cerebral territory failing to fill with carbon black was significantly smaller in the haemodiluted animals. This was true of each slice studied (table 2). In control animals at normal haematocrit the non-perfused area represented $23.05 \pm 2.25\%$ of the hemisphere when all nine coronal slices were considered together. In the haemodiluted animals the mean figure was $10.44 \pm 0.9\%$, a reduction of over 50% ($p < 0.001$). Furthermore, the area of failed perfusion was restricted to the anterior part of the hemisphere only, in haemodiluted animals.

The size of the ischaemic hemisphere was greater even at this early stage after middle cerebral artery occlusion. Of 45 brain slices measured in the control animals, all showed a greater area on the ischaemic side. Similarly all but 1 of 35 slices measured in the five haemodiluted animals showed the ischaemic side to be larger.

Glycerol

In a second series of experiments, 10 animals were again haemodiluted prior to middle cerebral occlusion. Five received an intravenous infusion of normal saline, and five 10% w/v glycerol in saline during the period of occlusion. The haematocrit fell to a comparable degree in both groups. Arterial gas analysis and pH levels were also comparable (table 3). No changes occurred in arterial blood pressure.

The area of non-filling of the middle cerebral vascular bed expressed as a percentage of hemisphere area averaged over nine coronal slices was $9.98 \pm 9.5\%$ in the haemodiluted, saline infused animals, and

Table 1 Physiological variables and haematocrit in haemodiluted and control rats

	Rats		
	Control (n = 6)	Haemodiluted (n = 5)	p. (Student <i>t</i>)
Haematocrit (per cent)	52.3 ± 1.6	30.4 ± 1.1	<0.001
pCO_2 (mm Hg)	31.7 ± 10	36.4 ± 1	NS
pH	7.29 ± 1.3	7.18 ± 0.07	NS
pO_2 (mm Hg)	131 ± 12	108 ± 2.4	<0.01

Table 2 Area of non perfusion as percentage of cross sectional area of hemisphere in coronal slices in haemodiluted and control rats

	Rats		
No. of slice (see Methods)	Control (n = 6)	Haemodiluted (n = 5)	p (Student <i>t</i>)
-4	38 ± 2.4	26.9 ± 2.4	<0.001
-3	32.5 ± 2.4	21.4 ± 3.0	<0.001
-2	27.3 ± 3.4	18.4 ± 1.5	<0.001
-1	24.3 ± 3.6	15.0 ± 2.0	<0.001
0	22.0 ± 3.1	10.7 ± 1.6	<0.001
+1	21.6 ± 3.0	0	<0.001
+2	17.8 ± 3.6	0	<0.001
+3	13.0 ± 1.3	0	<0.001
+4	10.7 ± 1.2	0	<0.001

Table 3 Physiological variables and haematocrit after haemodilution and infusion of saline or glycerol

	Rats		
	Haemodiluted (n = 5)	Haemodiluted + Glycerol (n = 5)	p (Student t)
Haematocrit (%)	40 ± 1.3	38.6 ± 3.0	NS
pCO ₂ (mm Hg)	34.2 ± 3.3	32.7 ± 5.0	NS
pH	7.25 ± 0.11	7.27 ± 0.06	NS
pO ₂ (mm Hg)	114.8 ± 13.6	109.7 ± 18	NS

13.65 ± 11.8% in the haemodiluted glycerol treated group. The difference is not significant. The figures for both these groups are comparable to those of the haemodiluted group in the first part of the study.

That glycerol had affected hemisphere swelling was obvious from the fact that the ischaemic hemisphere was the larger in 17 slices from treated brains, but the non-ischaemic side was larger in 18. This contrasts with the uniform finding of a swollen ischaemic side in control animals and those only haemodiluted (Part 1) (Chi square 26, p < 0.001).

Discussion

These results show that isovolaemic haemodilution to a haematocrit of 30.4 ± 1.1% in the rat at the time of middle cerebral occlusion reduces by 50% the area of the middle cerebral artery bed that is inaccessible to an intravascular marker. This is in keeping with earlier observations such as those of Sundt,¹³ who noted a potentially beneficial effect of haemodilution in comparable circumstances, though the actual levels of haematocrit were not published.

Although the dependence of blood flow on haematocrit (and viscosity) appears to be physiological and a reflection of the normal regulation of oxygen delivery,¹⁴ it is possible that flow in the maximally dilated ischaemic collateral bed could be limited by rheological factors. Such a dependence on haematocrit would explain the finding that the size of infarcts is related to the prevailing haematocrit level in the case of carotid occlusion.⁴

Recent pilot trials suggest that isovolaemic haemodilution may reduce the mortality and morbidity of acute ischaemic stroke in man, and multicentre trials are under way.¹⁵ It was noteworthy that despite reduction in the vascular territory not reached by an intravascular marker, after haemodilution, swelling of the infarcted hemisphere was still detectable.

An intravenous infusion of glycerol during the period of ischaemia apparently reduced such early ischaemic swelling but failed to make any detectable further improvement in vascular filling, though improvement in tissue perfusion has been shown by others.⁷

The results of this study suggest that hyperosmolar therapy might with advantage be combined with isovolaemic haemodilution in the treatment of acute cerebral ischaemia.

We are grateful to Mr TN Gajree and Miss E Weisblatt for skilled technical assistance.

References

- Kannel WB, Gordon T, Wolf PA, McNamara P. Haemoglobin and the role of cerebral infarction. The Framingham Study. *Stroke* 1972;3:409-20.
- Toghi H, Yamamuchi H, Murakami M, Kameyama M. Importance of haematocrit as a risk factor in cerebral infarction. *Stroke* 1978;9:369-74.
- Forbes CD, Lowe GDO. Effect of high haematocrit on acute mortality in stroke. *Clin Hemorheol* 1983;3:308.
- Harrison MJG, Pollock S, Kendall BE, Marshall J. Effect of haematocrit on carotid stenosis and cerebral infarction. *Lancet* 1981;2:114-5.
- Gottstein U. Haemodilution: Therapy in acute ischaemic stroke. In: *Cerebral Ischaemia*. Bes A, Braquet P, Paoletti R, Siesjo BK, eds. Amsterdam Elsevier Holland, 1984 311-24.
- White OB, Norris JW, Hachinski VC, Lewis A. Death in early stroke, causes and mechanisms. *Stroke* 1979;10:743.
- Hossmann KA, Matsuoka Y, Bloink M, Fitzgerald G, Hossmann V. Treatment of experimental infarction in the cat brain. In: *Drugs and Methods in Cardiovascular disease*. Cahn J, ed. Paris: Pergamon Press, 1981:427-34.
- Mulley G, Wilcox FG, Mitchell JRA. Dexamethasone in acute stroke. *Br Med J* 1978;2:994-6.
- Hachinski V, Norris JW. Steroid therapy in ischaemic stroke. *Can J Neurol Sci* 1984;11:321.
- Matthew NT, Meyer JS, Rivera VM, Charney JZ, Hartman A. Double-blind evaluation of glycerol therapy. *Lancet* 1972;2:1327-9.
- Tamura A, Graham DI, McCulloch J, Teasdale GM. Focal Cerebral Ischaemia in the Rat. 1. Description of technique and Early Neurophysiological Consequences following Middle Cerebral Artery Occlusion. *J Cerebr Blood Flow Metabol* 1981;1:53-60.
- Harrison MJG, Arnold J, Sedal L, Ross Russell RW. Ischaemic swelling of cerebral hemisphere in the gerbil. *J Neurol Neurosurg Psychiatry* 1975;38:1194-6.
- Sundt TM, Waltz AG, Sayre AG. Experimental infarction: Modification by treatment with haemodiluting, haemoconcentrating and dehydrating agents. *J Neurosurg* 1966;26:45-56.
- Brown MN, Wade JPH, Marshall J. Fundamental importance of Arterial Oxygen Content in the Regulation of Cerebral Blood Flow in Man. *Brain* 1985;108:81-93.
- Strand T, Asphund K, Erikssen S, Hagg E, Lithner F, Wester PO. A randomised controlled trial of haemodilution therapy in acute ischaemic stroke. *Stroke* 1985;15:980-9.